IMPROVED FLUORIMETRIC ASSAY OF PLASMA PROPRANOLOL

Propranolol is commonly assayed using the fluorimetric method reported by Shand, Nuckolls & Oates (1970). Other similar methods (Black, Duncan & Shanks, 1965, Potter, 1967, Lameijer, 1973) seem to be less accurate and practical, and they carry an additional toxicological risk because benzene is used as an extraction solvent.

Recently the accuracy and sensitivity of the Shand *et al.* (1970) method has been criticized by Chidsey, Morselli, Bianchetti, Morganti, Leonetti & Zanchetti, (1975), because the g.l.c. method using electron capture detection described by Disalle, Baker, Bareggi, Watkins, Chidsey, Frigerio & Morselli, (1973), was alleged to have a limit of detection well below 5 ng/ml in 0.5 ml plasma samples, whereas the use of the Shand *et al.* (1970) method often resulted in erratically high plasma blanks, besides not being reproducible below a level of 30 ng/ml in a 4 ml sample. We tend to agree with these objections after comparing the two methods. However, the g.l.c. assay is exceptionally time-consuming and necessitates the use of sophisticated and expensive equipment. Nevertheless, by introducing minor modifications, the fluorimetric method can be made nearly as sensitive and accurate as the g.l.c. electron captive detection method.

The following reagents were used: hexane (für die Fluoreszenzspektroskopie, Merck); sodium hydroxide pellets, 37% hydrochloric acid (analytical grade, Merck); isoamylalcohol (für die Fluoreszenzspektroskopie, Merck); propranolol hydrochloride (ICI Pharmaceuticals Ltd, Macclesfield). All reagent solutions were made up in AnalaR grade water (British Drug Houses Ltd). All glassware was soaked overnight in 20% nitric acid, washed with double-distilled water, and dried for 8 h at 90° C. A propranolol standard solution (100 ng/ml) was made up in 0.01 N hydrochloric acid. Heparinized human plasma (1 ml) was pipetted into 30 ml glass-stoppered round-bottom Quickfit centrifuge tubes, made alkaline with 2 N sodium hydroxide solution (0.1 ml) and extracted with hexane (5 ml) containing 1.5% (v/v) isoamylalcohol during 15 min in a Griffin flask shaker. The aqueous and organic layers were separated by spinning in a Sorvall RC-3 centrifuge during 5 min at 3000 rev/min and 4° C; 4 ml of the organic supernatant, using a 5 ml adjustable Oxford pipette, was quickly transferred to a clean tube, and the extraction procedure repeated with a second volume of the hexane/isoamylalcohol mixture. To the pooled supernatants (8 ml) 0.01 N hydrochloric acid (3 ml) was added. After shaking for 15 min the mixture was centrifuged during 5 min at 3000 rev/min and 4° C, and organic supernatant removed as completely as possible by aspiration. Actual fluorescence measurements were done in 1 cm QS-cuvettes (Hellma) in the Aminco-Bowman SPF at emission and excitation wavelengths of 290 and 352 nm respectively, using 3 mm slits.

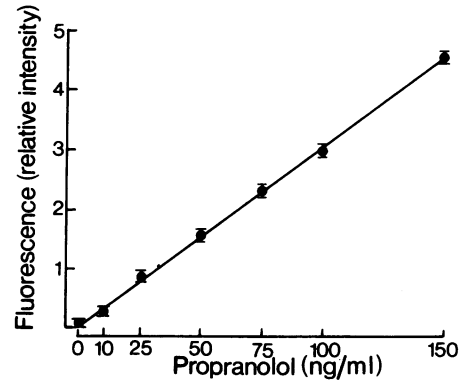

Figure 1 Standard curve, (mean ± s.d.), propranolol added to plasma, 0-150 ng/ml, $r = 0.9995$.

A standard curve obtained for 0-10-25-50-75-100-150 ng/ml propranolol is shown in Figure 1. No statistically significant differences were found between plasma and reagent (0.01 N hydrochloric acid) blanks, whether or not taken through the complete assay. Using this procedure the mean coefficient of variation over the whole range of standard samples (propranolol added to plasma) was 7.4 ± 3.0%. Therefore an acceptable accuracy of the assay can be expected down to a plasma concentration of 5 ng/ml. Hexane, having a lower boiling point than heptane, proved to be the most suitable solvent, because the samples used in the final fluorimetric assay were less easily contaminated by light-scattering droplets or the deposition of a solvent film on the inside of the cuvette, a problem which, by our experience, often invalidated the results obtained with assay methods using benzene as the extraction solvent. No hexane was lost during the extraction procedure because samples were spun at 4° C. The addition of isoamylalcohol as a surfactant, as proposed by Shand *et al.* (1970),

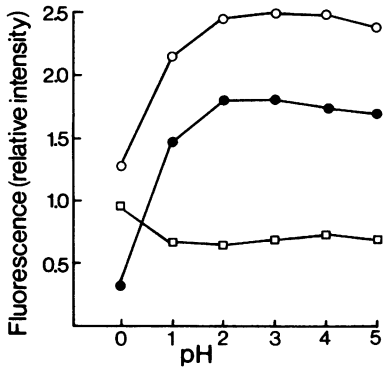

Figure 2 pH–dependence of propranolol fluorescence; plasma blank (□), propranolol (15 ng/ml), added to plasma (○) and propranolol (15 mg/ml) added to plasma, corrected for blank (●).

proved to be essential because it enhances the efficiency of the extraction from 75 to 94%. Double hexane extraction was chosen in order to improve extraction ratio and efficiency, and to minimize pipetting errors. Efficiency was not improved by prolongation of any extraction time over 15 min. Extraction with 0.01 N hydrochloric acid is preferable, because higher fluorescence values are obtained, which are not affected by minor changes in pH (Figure 2). Theoretically extraction at pH 3, as in Potter's (1967) assay, would be optimal. However, the instability of dilute acetic acid at room temperature is a major disadvantage.

Propranolol shows maximal fluorescence at excitation and emission wavelengths of 292 and 348 nm respectively. Minimal interference from the peak at 310 nm observed in all plasma blanks on the one side, and inaccuracy through measuring on the steep slope of the emission peak of propranolol at 360 nm on the other hand, was found at an emission wavelength of 352 nm. The modifications proposed make the assay applicable to plasma samples of 0.5-1.0 ml with a sensitivity and accuracy approaching the results of the g.l.c. method (diSalle *et al.*, 1973).

L. OFFERHAUS & J.R. VAN DER VECHT

Clinical Pharmacology Unit, Department of Medicine, University Hospital (Wilhelmina Gasthuis), Amsterdam-1013, Holland

Received April 26, 1976

References

BLACK, J.W., DUNCAN, W.A.M. & SHANKS, R.G. (1965). Comparison of some properties of pronethalol and propranolol. *Br. J. Pharmac.*, **25**, 577-591.

CHIDSEY, C.A., MORSELLI, P., BIANCHETTI, G., MORGANTI, A., LEONETTI, G. & ZANCHETTI, A. (1975). Studies of the absorption and removal of propranolol in hypertensive patients during therapy. *Circulation*, **52**, 313-318.

diSALLE, E., BAKER, K.M., BAREGGI, S.R., WATKINS, W.D., CHIDSEY, C.A., FRIGERIO, A. & MORSELLI, P.L. (1973). A sensitive gas chromatographic method for the determination of propranolol in human plasma. *J. Chromatogr.*, **84**, 347-353.

LAMEIJER, W. (1973). *Kinetische en farmacologische aspecten van kinidine en propranolol met betrekking tot geisoleerde hartspierpreparaten.* Thesis, University of Amsterdam. De Kroon, Hilversum.

POTTER, L.T. (1967). Uptake of propranolol by isolated guinea-pig atria. *J. Pharmac. exp. Ther.*, **155**, 91-100.

SHAND, D.G., NUCKOLLS, E.M. & OATES, J.A. (1970). Plasma propranolol levels in adults with observations in four children. *Clin. Pharmac. Ther.*, **11**, 112-120.

A RAPID METHOD FOR THE DETERMINATION OF DIPHENHYDRAMINE IN PLASMA

The antihistamines are one of the most widely used groups of drugs. However because of the very low plasma levels found after normal doses of these drugs little is known about their disposition in man. In order to investigate the disposition of the ethanolamine group of antihistamines in man, an assay capable of determining these drugs in the concentration range of 10-200 ng ml^{-1} in the presence of its metabolites was required. Glazko, Dill, Young, Smith & Ogilvie (1974) reported a fluorimetric method for the estimation of diphenhydramine, a member of the ethanolamine group, which involved several steps including an oxidation. Diphenhydramine has also been estimated by g.l.c. However, the method of Bilzer & Gundert-Remy (1973) does not distinguish between diphenhydramine and its metabolites and is subject to interference from plasma constituents, the g.l.c. method of Albert, Sakmar, Morais, Hallmark & Wagner, (1974) like the method of